**NUTRITIONAL, ANTIDIABETIC, and ANTILIPIDEMIC STUDIES of ETHANOLIC EXTRACT of P. TUBER-REGIUM in HIGH-FAT-STREPTOZOTOCIN-INDUCED DIABETIC MALE WISTAR ALBINO RATS**

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ABSTRACT

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| *Pleurotus tuber-regium*, a nutritionally dense and bioactive-rich edible mushroom prevalent in West Africa, is gaining attention for its potential role in functional foods and complementary strategies for managing metabolic diseases. This study investigated the nutritional composition and some therapeutic potential of *P. tuber-regium* in high-fat diet and streptozotocin (STZ)-induced diabetic male Wistar albino rats. Proximate analysis was assayed using the methods described in AOAC, 2019, revealing the *P. tuber-regium* to be rich in carbohydrates (68.04%), crude protein (19.92%), and dietary fibre (5.44%), with low fat content (0.97%) and minimal moisture (2.20%), highlighting its suitability for hypocaloric diets. High-performance liquid chromatography (HPLC) analysis showed the presence of essential B-complex vitamins—thiamine (0.006 mg/g), riboflavin (0.003 mg/g), niacin (0.001 mg/g)—and vitamin C (0.008 mg/g), suggesting antioxidant and metabolic support roles. Mineral profiling via inductively coupled plasma-optical emission spectroscopy (ICP-OES) further confirmed the presence of micronutrients essential for enzymatic functions and metabolic homeostasis.  Following a 9-week oral administration of *P. tuber-regium* ethanol extract at graded doses and metformin (200, 400, 800, 80 mg/kg respectively), diabetic rats demonstrated significant (p < 0.05) improvements in fasting blood glucose, lipid profile (triglycerides, total cholesterol, High Density Lipoprotein, Low Density Lippprotein), and pancreatic amylase activity. The Histomorphology assessments of pancreatic tissues also revealed restoration of islet architecture in extract-treated groups. Acute oral toxicity testing indicated no mortality or observable toxicological symptoms up to 6000 mg/kg, confirming the safety of *P. tuber-regium* extract.  The results suggest that *P. tuber-regium* possesses promising antidiabetic and hypolipidemic properties, potentially mediated by its fibre content, micronutrients, and bioactive constituents. This supports its application as a nutraceutical in the dietary management of type 2 diabetes Mellitus. An extended mechanistic and clinical studies are warranted to elucidate the pathways involved and to validate translational applicability in human populations.  **Keywords:** P. *tuber-regium,* lipid profile, nutraceutical, Antidiabetic effect, lipid profile. |

1. INTRODUCTION

Edible mushrooms are increasingly being investigated for their nutritional and health benefits (Khan and Tania, 2012). The oyster mushroom (Pleurotus spp.) is one of the most widely cultivated edible mushrooms and is well-known for its therapeutic properties, culinary applications, and uses in biotechnology, environmental management, and pharmaceuticals (Rathod *et al*., 2021; El-Ramady *et al*., 2022; Assemie & Abaya, 2022). These remarkable fungi also grow in the wild, serving as food for both animals and humans (Amara & El-Baky, 2023; Case *et al*., 2022). They are crucial in maintaining ecosystem balance, act as raw materials for various industries, and serve as ingredients in traditional medicines (Bell *et al*., 2022; Panda *et al*., 2024; Ogwu *et al*., 2025). Törős *et al.,* 2022; Carrasco *et al.,* 2021; Adedokun *et al*., 2022 reported that oyster mushrooms are the most commercially cultivated globally. In Nigeria, among the numerous Pleurotus species, P. tuber-regium—commonly known as the tiger milk mushroom—holds a special place in traditional diets, local snacks, as a soup thickener, meat substitute, and bulking agent (Kumar & Netam *et al., 2022*; Afolabi *et al*., 2024; Abdullah *et al.,* 2024). This fungus has gained increasing recognition in recent years, accounting for over 25% of all commercially grown edible mushrooms, due to its ability to grow on agro-waste and its rich nutritional profile (Raman *et al.,2021;* Ghafoor, Niazi *et al*.,2024) P. tuber-regium is regarded as a valuable source of essential amino acids, fat- and water-soluble vitamins, carbohydrates, proteins, dietary fiber, and minerals (Kolawole *et al., 2021;* Raman *et al.,2021)*. Studies have shown that *P. tuber-regium* is a reservoir of both micro- and macronutrients (Igbokwe *et al.,2015;* Lin *et al., 2020; Ekute* & Nwokocha, 2021).

Its amino acid profile, low fat content, vitamin composition, and high dietary fibre make it suitable for managing metabolic disorders such as diabetes, hypertension, and cardiovascular diseases (Adeyi *et al*., 2021). This natural resource is also rich in essential minerals such as calcium, phosphorus, potassium, magnesium, iron, and zinc, which serve as cofactors for enzymes involved in various metabolic processes and immune modulation. It plays a role in blood glucose and lipid regulation as well as the maintenance of cellular function (Ijeh *et al*.,2009; Adeyi *et al*., 2021). Furthermore, P. tuber-regium contains a wide variety of bioactive compounds, including β-glucans, terpenoids, steroids, and phenolics, which exhibit antimicrobial, immunomodulatory, and antioxidant Properties (Nworu *et al., 2015;* Alaribe *et al.,2018;* Lin *et al., 2020)*. Recently, there has been a growing interest in sustainable, non-animal-based nutrition. With its dense nutritional content, P. tuber-regium presents a promising alternative source of nutrition and a potential functional food ingredient (Lin *et al., 2020;* Adeyi *et al*., 2021; Aswathy *et al., 2024)*.

Metabolic disorders such as diabetes mellitus, hypercholesteremia have been a major public health burden in recent times (Igbokwe *et al.,*2015*)*. It occurs from complications as a result of chronic persistent hyperglycemic condition. These conditions have been managed with several interventions which presence residual side effect (Adeyi *et al*., 2021). Recently, there is a growing interest in dietary and lifestyle management of these metabolic disorders with low or no side effects (Barrea *et al,* 2023; Azmat *et al*, 2025). This study explores the nutritional and bioactive profile of *P. tuber-regium* and its effects on blood glucose levels, lipid profile, pancreatic amylase activity, and pancreatic histomorphology in high-fat-streptozotocin-induced diabetic Wistar albino rats. The findings are expected to contribute to the scientific validation of *P. tuber-regium* as a functional food in the dietary management of diabetes.

1. material and methods
   1. **Collection of mushrooms**

This was done using the modified method as described by Okorie et al. 2021. *P. tuber-regium* is one of the most popular edible mushrooms and can be easily cultivated on various agricultural wastes such as straw, sawdust, coffee grounds, cotton waste, and other lignocellulosic substrates. Composed substrates made from a mix of sawdust and rice bran (9:1) were placed into polyethylene bags, which were sterilized in an automated autoclave at 121°C to eliminate competing microorganisms. These substrates were allowed to cool to ambient temperature before being inoculated with the spawns of *P. tuber-regium* cultivated in the mushroom centre of the Federal Institute of Industrial Research, Oshodi. Inoculation was performed under aseptic conditions. The incubation stage was conducted in darkness to enable mycelium growth through the substrate, forming a network. Fully ramified substrates (17-22 days) were exposed to appropriate environmental conditions (light, humidity, temperature) to induce fruiting and the development of mushroom caps. The mature oyster mushrooms were harvested by cutting clusters at the base of the stem.

**2.2 Nutritional compositions**

The nutritional composition of *P. tuber-regium* was evaluated in this study. The Proximate analysis, vitamins, and mineral elements.

**2.2.1 Proximate analysis**

Proximate analysis plays a vital role in determining the nutritive values of various food substances. It serves as a foundational tool for the development of new food products, assessment of animal feeds, research and development activities, quality assurance, and compliance with regulatory standards. This analytical method is widely applied in the food industry, animal nutrition sector, and research institutions to investigate the properties and behaviour of different materials. It facilitates the optimization of food processing, enhances product safety, and supports accurate nutritional labelling. In this study, proximate composition analysis was performed to quantify the moisture, ash, crude protein, crude fat, and fibre contents, following the procedures outlined by the Association of Official Analytical Chemists (AOAC, 2019).

* + 1. **Determination of Crude Protein**

The Kjeldahl method (1965) was used to determine the crude protein content of the edible mushroom sample due to its accuracy and aptness for nitrogen analysis. In this procedure, the mushroom sample was digested with concentrated sulfuric acid (H₂SO₄), which decomposed the organic matter and converted nitrogen-containing compounds into ammonium sulfate. The digest was subsequently neutralized with sodium hydroxide (NaOH), releasing ammonia gas (NH₃) during steam distillation. The liberated ammonia was captured in a known volume of boric acid solution. The amount of ammonia absorbed was then quantified by titration with a standardized hydrochloric acid (HCl) solution. The nitrogen content thus obtained was converted to crude protein using a standard conversion factor of 6.25, applicable to most organic materials.

* + 1. **Determination of Crude fibre**

The FOSS Fibertec-2010 apparatus was employed to analyse crude fibre in mushroom samples. The equipment is automated and standardizes the process, ensuring the accuracy and reproducibility of the results obtained*. This process involves a systematic and sequential extraction of fibre components from a sample using chemical treatments, filtration, washing, drying, and weighing to determine the crude fibre content.*

* + 1. **Evaluation of Moisture Content**

The AND-MS70 moisture analyser was utilized to measure the moisture content of the dehydrated mushroom samples. This parameter is essential for maintaining quality control in processed food products and for complying with standards. This method involves evaporating moisture from a sample using halogen heating, resulting in weight loss in the analysed sample. The weight change is expressed as a percentage of the initial weight to determine the moisture content.

* + 1. **Determination of Fat**

The Soxhlet extraction method is a reliable and efficient approach for fat analysis in specified samples. A thimble made of glass or cellulose is used to hold the sample, and a suitable organic solvent such as petroleum ether or hexane is selected based on its ability to effectively dissolve lipids. Additionally, the boiling point of the solvent must be low.

* + 1. **Determination of Ash Content**

The Carbolyte-AAF1100 furnace was used to determine the ash content. This was achieved by heating the dried sample to a temperature between 500-600°C, which incinerates all organic matter and leaves only the inorganic ash. After cooling the inorganic material in a desiccator, it is weighed to calculate the percentage of ash.

* 1. **Determination Of Minerals**

Inductively coupled plasma (ICP) was employed to evaluate the mineral content in the mushroom samples. This approach allows for multi-element analysis, showcasing high sensitivity and accuracy in elemental detection. The Agilent 5800 VDV ICP-OES [Agilent Technologies USA] was equipped with a Sea-Spray nebulizer, a double-pass glass cyclonic spray chamber, and a semi-demountable dual view (DV) injector torch with a 1.8 mm inner diameter. The Agilent SPS 4 autosampler facilitated the rapid and automated sample delivery to the ICP-OES, as outlined in the method of US EPA 200.7.

* 1. **Vitamin Analysis**

High Performance Liquid Chromatography (HPLC) is a common method for analysing a range of compounds, including vitamins. The HPLC system requires an appropriate column, detector, mobile phase, and pumps, which manage the flow rate of the solvent containing the analytes. Before injection, analytes must be concentrated to eliminate possible interfering substances, typically involving processes like extraction, filtration, and dilution. Generally, HPLC techniques for vitamin analysis guarantee accurate, precise, specific, and valid results. Different concentrations of these standards were tested under the same chromatographic conditions as the target compounds, resulting in a calibration curve for each standard.

* 1. **Mushroom Collection and Drying**

Mushroom samples were collected from the biotechnology department of the Federal Institute of Industrial Research Oshodi, Lagos, where oyster mushrooms are cultivated. Mushrooms were dehydrated using a stainless dehydrator (ST-02, 220- 240V, 50Hz, 1500W) at a temperature of 45°C to a constant weight and pulverized with an electric blender (Kenwood blender 3.0L, 8500W, KC-241B, UK-SPEC) and packed with zip-lock bags for further applications.

* 1. **Extraction Of Mushroom**

The extract preparation was performed using a modified method described by Soares *et al*. (2009). The powdered mushroom sample (500g) was macerated in 2.5 L of absolute ethanol (98.9%) for four days. The resulting mixture was filtered through a clean white muslin cloth, and the filtrate was concentrated at 40°C using a rotary evaporator. The final yield of the extract was 40 g, corresponding to a percentage yield of 12%.

* 1. **Toxicity Study of Mushroom Extract (LD50)**

An acute oral toxicity study (LD₅₀) was conducted following the method described by Obode et al. (2021). Groups of eight Wistar rats were administered extract doses of 1500, 3000, and 6000 mg/kg body weight, respectively. No mortality or observable signs of toxicity were recorded during the study, indicating that the extract was non-toxic at the administered doses.

* 1. **Animals Used in Experiment**

Healthy male albino Wistar rats were housed under standard laboratory conditions at an ambient temperature and maintained on a 12-hour light/dark cycle. During a 72-hour acclimatization period, the rats were fed standardized laboratory chow obtained from Ojuoye Market, Mushin, Lagos, and provided with water ad libitum. The study was carried out with the guidelines approved for the Care and Use of Laboratory Animals by Rhema University, Aba, Abia State.

* 1. **Induction Of Diabetes**

Diabetic conditions were induced by a single intraperitoneal injection (i.p) of 40mg/kg of streptozotocin in about 6 mL of citrate buffer (pH 4.5) and monitored. After 48 hours of streptozotocin injection, some physiological observations were seen, such as frequent urination of experimental animals as reported by Longhurst & Belis.,1986; Akbarzadeh *et al*.,2007. Then an *On Call® Plus II* glucometer with blood glucose test strips (Acon Laboratories, San Diego, USA) were used to check the blood glucose of STZ-induced animals, with serum glucose levels above 200mg/dl considered as diabetic and separated and grouped for treatment.

* 1. **Experimental Design for Study**

Following acclimatization, the animals designated for diabetes induction were fed a high-fat diet and high-fructose syrup for 18 days to promote insulin resistance. The diabetic rats were randomized into five treatment groups (n = 10 per group), and a sixth group served as the normal control. The experimental groups were as follows:

* **Group 1 (Normal Control, NC):** Non-diabetic rats fed standard rat chow and water *ad libitum*
* **Group 2 (Diabetic Control, DC):** Diabetic rats receiving no treatment
* **Group 3 (PTE):** Diabetic rats treated with 200 mg/kg of *Pleurotus tuber-regium* extract
* **Group 4 (PTM):** Diabetic rats treated with 400 mg/kg of *Pleurotus tuber-regium* extract
* **Group 5 (PTH):** Diabetic rats treated with 800mg/kg of *Pleurotus tuber-regium* extract
* **Group 6 (STD):** Diabetic rats treated with 80mg/kg of Metformin

Treatments were administered orally once daily for 9 weeks (63 days). All rats had continuous access to standard chow and water throughout the study. Body weight and fasting blood glucose levels were monitored regularly during the experimental period.

* 1. **Determination of Fasting Blood Glucose**

Fasting blood glucose levels were determined after a 12-hour overnight fast. A small puncture was made at the tip of each rat’s tail, and blood samples were collected using *On Call® Plus II* blood glucose test strips (Acon Laboratories, San Diego, USA). The strips were inserted into the *On Call® Plus II* glucometer to obtain glucose readings, following the method described by Jarald et al. (2013). Blood glucose measurements were taken before the commencement of treatment and subsequently at 7-day intervals throughout the experiment. Following 48 hours of streptozotocin (40 mg/kg) administration, blood samples were collected for serum assay, and the pancreas was harvested for further biochemical and histological analyses.

* 1. **Determination of Blood Lipid Profile**

Blood samples were collected from the rats via the retro-orbital plexus under light anaesthesia. The samples were allowed to clot at room temperature and subsequently centrifuged at 3000 rpm for 15 minutes to separate the serum. Lipid profile analysis was conducted using the serum obtained. Triglycerides (TG), total cholesterol (TC), and high-density lipoprotein cholesterol (HDL-C) levels were determined using a Biolab assay kit with an automated chemistry analyser (SFRI, BSA, France), following the procedure described by Gebrie et al. (2018), with slight modifications in the analyser model employed. Low-density lipoprotein cholesterol (LDL-C) was calculated using the Friedewald equation (Friedewald & Levy, 1972)

LDL Cholesterol = Total Cholesterol – Triglyceride – HDL Cholesterol

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* 1. **Determination of Pancreatic Amylase Activity**

Pancreatic amylase is an enzyme secreted by the pancreas that plays a vital role in the digestion of carbohydrates. Blood samples were collected from the animals in EDTA-treated tubes and centrifuged to separate the plasma from the serum. Pancreatic amylase was subsequently extracted using appropriate methods, such as ultracentrifugation, gel filtration, or affinity chromatography, as described by Stiefel and Keller (1973).

* 1. **Statistical Analysis**

Statistical analysis was performed using GraphPad Prism version 5.0d (GraphPad Software, San Diego, CA, USA). Differences between the mean values of the experimental groups were evaluated using one-way analysis of variance (ANOVA), followed by Tukey’s post hoc multiple comparison test. Data are presented as mean ± standard error of the mean (SEM). A *p*-value=0.05 was considered statistically significant.

3. results and discussion

**3.0. RESULTS**

**3.1. PROXIMATE ANALYSIS**

**Table1: Proximate composition of *Pleurotus tuber-regium* (% dry weight basis)**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Component** | **Moisture** | **Crude protein** | **Crude fibre** | **Crude fat** | **Ash** | **Carbohydrate** |
| ***P. tuberregium* (%)** | 2.20±0.03 | 19.923±0.06 | 5.44±0.003 | 0.97±0.03 | 3.44±0.02 | 68.04±0.012 |
| **Method Used (AOAC 2019)** | AOAC 925.10 | AOAC 920.87 | AOAC 991.43 | AOAC 991.36 | AOAC 923.03 | By difference |

\*Values are mean ± standard deviation of triplicate determinations. *Carbohydrate calculated by difference: 100 - (protein + fat + fibre + ash + moisture).*

**3.2. VITAMIN ANALYSIS**

**Table 2: Vitamin Content of *Pleurotus tuber-regium***

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **VITAMIN** | **AMOUNT** | **RDI** | **UNIT** | **METHOD OF ANALYSIS** |
| **Vitamin A(Retinol)** | ND | 0.99 | mg/g | **Agilent 1100 HPLC (Van Wayenbergh *et al 2020*)** |
| **Vitamin B1(Thiamine)** | 0.006 | 1.2 | mg/g | **Agilent 1100 HPLC (Patle *et al 2022*)** |
| **VitaminB2 (Riboflavin)** | 0.003 | 1.3 | mg/g | **Agilent 1100 HPLC (**AOAC 2019 and Ph. Eur 2017) |
| **Vitamin B3(Niacin)** | 0.001 | 15 | mg/g | **Agilent 1100 HPLC (Patle *et al 2022*)** |
| **Vitamin B5(Pantothenic acid)** | ND | 5 | mg/g | **Agilent 1100 HPLC (**AOAC 2019 and Ph. Eur 2017) |
| **Vitamin B6 (Pyridoxine)** | ND | 1.3 | mg/g | **Agilent 1100 HPLC (Patle *et al 2022*)** |
| **Vitamin B9 (Folate)** | ND | 0.5 | mg/g | **Agilent 1100 HPLC (**AOAC 2019 and Ph. Eur 2017) |
| **Vitamin B12(Cyanocobalamin)** | ND | 0.002 | mg/g | **Agilent 1100 HPLC (**AOAC 2019) |
| **Vitamin C (Ascorbic acid)** | 0.008 | 90 | mg/g | **Agilent 1100 HPLC (Patle *et al 2022*)** |
| **Vitamin D (Cholecalciferol)** | 0.07 | 0.21 | mg/g | **Agilent 1100 HPLC (**AOAC 2019) |
| **Vitamin E (Tocopherol)** | 0.013 | 15 | mg/g | **Agilent 1100 HPLC (**AOAC 2019) |

**Recommended Daily Intake (RDI) values are expressed on a per-day basis. Sources: Food and Agriculture Organization (FAO, 2023); and Codex Alimentarius reference standards.**

**3.3. MINERAL ANALYSIS**

**Table 3 : Mineral analysis of *P. tuberregium***

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Component** | **Calcium** | **Magnesium** | **Potassium** | **Iron** | **Zinc** | **Sodium** | **Manganese** |
| ***P. tuberregium* (%)** | 797.75  ±5.25 | |  | | --- | | 151.18  ±5.18 | |  | | 3826.86  ±9.51 | 2.70  ±0.10 | 1.88  ±0.06 | 156.8  ±3.34 | 0.16  ±0.07 |
| **Method Used/Instrument** | **ICP instrumentation/The Agilent 5800 VDV ICP-OES** | | | | | | |

**3.4. HYPOGLYCEMIC EFFECTS OF ETHANOLIC EXTRACT OF *P. tuberrigium***

**3.4.1. Change in Body Weights**

The effect of different doses of the ethanolic fraction of *P. tuberigium* on the body weight of normoglycemic and STZ-diabetic rats was observed for a period of 9 weeks, to ascertain a long-term effect of treatment on the body weight of experimental animals. Figure 1 and 2 shows the body weight changes in experimental animals for 28 days and 63 days.



Fig .1,2. Change in Body Weights in *P. tuberrigium*

**3.4.2. FASTING BLOOD SUGAR**

The fasting blood glucose in the normoglycemic and diabetic rats were evaluated and showed a significant decrease in the glucose levels, after 9 weeks(63days) of the experiments. The PTE-78.2%, PTM-88.4%, PTH-79.0% and STD-79.37%.

**Fig.3,4. Bar graph showing fasting blood sugar concentration**

**3.4.3. PANCREATIC AMYLASE**

Pancreatic amylase is an enzyme produced in the pancreas that catalyses the breakdown of carbohydrates into smaller simple sugars like maltose and dextrin. In the study of diabetes, pancreatic amylase is of interest owing to its effect on postprandial glucose levels which may indicate an underlying pancreatic dysfunction. Monitoring the pancreatic amylase levels can provide insight for therapeutic interventions aimed in diabetic management. In this study pancreatic amylase was evaluated in normoglycemic and the STZ-induced experimental animals. From figures below, it was observed that at 28 day, groups PTE (200mg/kg), PTM (400mg/kg) and STD (80mg/kg) had increased pancreatic amylase production while PTH (800mg/kg) was slightly significantly decreased compared to the normoglycemic animals. Furthermore, extended treatment for 63days, regulated the release of pancreatic amylase from the pancreas, therefore regulating the absorption of glucose to the cells, as could be related with the blood glucose values seen on the glucometer.

**Fig.5,6. Bar graph showing pancreatic amylase activity**

**3.5. CHOLESTEROL DETERMINATION**

The blood cholesterol (mg/gl) of the treatment groups is presented in Figures 7 and 8. It was observed that at 28 days, there was no significant difference in NC, PTE, and PTH, while there was a decrease in total cholesterol(P<0.01) in all treatment groups of varied doses of ethanolic extract and standard drug (STD)- metformin when compared to NC.



Fig.7,8. Bar graph showing cholesterol determination

The HDL levels (mg/dl) of animals in the normal and treatment groups are presented in Figures 9 and 10. It shows that after 28 days of study, there was a significant difference(P<0.05) in PTM, PTH, and STD compared to the normal control (NC). However, a 9-week study revealed a decrease in the HDL levels (P<0.001) in PTH and STD, while PTE and PTM remained comparable to NC.

Fig.9,10. Bar graph showing HDL levels

The triglyceride levels (mg/dl) of animals in the normal and treatment groups are presented in Figures 11 and 12. Groups NC, PTE, and PTH were comparable, while there was a significant decrease (P<0.01) in group PTM and STD. At 63 days of the study, there was an increase in PTH, then PTE, PTM, and STD was comparable to the normal control.

Fig.11,12. Bar graph showing triglyceride levels

Fig 13, LDL in group PTH increase significantly (p<0.001) in PTH, while Fig 14 showed a decrease in PTH (P<0.05) and STD (P<0.01) compared to NC.

Fig. 13,14. Bar graph showing LDL in group PTH

**HISTOPATHOLOGY**

The pathology of the pancreas was investigated to assess the effects of the treatments on the integrity of the pancreas with a focus on potential tissue damage. This is to further corroborate the findings observed in lipid profiles, blood glucose levels, and pancreatic amylase assays. Figure 13 shows sections of the pancreatic tissues examined using hematoxylin and eosin (H&E) staining at X100 magnification. The pancreatic tissue sections at 28 days showed a mild to moderate distortion of exocrine acini and islet of Langerhans with signs of cellular degeneration, necrosis, or inflammatory infiltration in PTE, PTM, and PTH. At 63 days, the morphology of tissues shows no abnormality or inflammation.

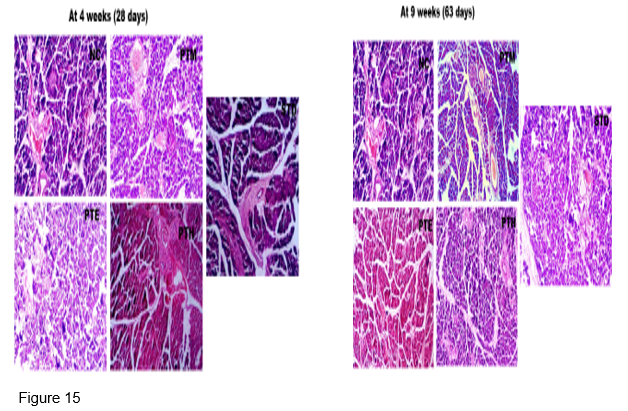


Fig. .15 Histopathology of the pancreas

The figures show the X 100 H& E stain of pancreatic tissues of the normoglycemic and diabetic treatment groups of three doses of ethanolic extract compared with standard drug (metformin). Histologic section of tissue NC and STD for both 28days and 63 days shows normal appearing exocrine acini with, no shrinkage of islets of Langerhans nor degeneration and necrosis of its component’s cells. For PTE, PTM and PTH at 28 days, there was mild to moderate distortion of exocrine acini and islet of Langerhans with signs of cellular degeneration. Meanwhile, at 63 days the pancreatic tissues morphology could be comparable to the normoglycemic group, revealing no inflammation or abnormality in pancreatic tissues.

**DISSCUSSIONS**

The global financial burden associated with the treatment of metabolic disorders, particularly those involving pancreatic β-cell dysfunction, remains substantial (Abdalla, 2024; Saleem *et al*, 2024). In light of this, growing attention has been directed toward the use of natural products with minimal adverse effects as therapeutic alternatives. This study explored the nutritional composition and therapeutic potential of an ethanolic extract of *Pleurotus tuber-regium* (P. tuber-regium) in high-fat diet and streptozotocin (STZ)-induced diabetic male Wistar rats, emphasizing its antidiabetic, hypocholesterolemic, and pancreatic enzyme-modulating properties. Proximate analysis revealed that *P. tuber-regium* is a nutrient-dense mushroom, with high levels of carbohydrates (68.04%), crude protein (19.92%), and dietary fiber (5.44%), corroborating previous reports on its nutritional richness (Chen & Cheung, 2014; Oranus *et al*, 2014; Igbokwe *et a*l, 2015). These macronutrients are essential for energy metabolism, glycemic control, and gastrointestinal health (Lin *et al*, 2020; Mo *et al*, 2024). The composition supports the mushroom’s traditional role as a meat substitute and dietary bulking agent, particularly in Nigerian cuisine. Vitamin profiling indicated the presence of both fat- and water-soluble vitamins in relatively low concentrations, including vitamin D (0.07 mg/g), vitamin E (0.013 mg/g), and trace amounts of thiamine (B1), riboflavin (B2), and ascorbic acid (vitamin C). Despite their modest levels, these vitamins may act synergistically to support antioxidant defense mechanisms and immune modulation (Boa, 2004; Jayachandran *et al*, 2017; Majesty *et al*, 2018). The mineral content of *P. tuber-regium* was notably high in potassium (3826.86 mg/100g), calcium (797.75 mg/100g), magnesium (151.18 mg/100g), and sodium (156.80 mg/100g), all of which are critical regulators of insulin signaling, cellular metabolism, neural conduction, and cardiovascular function (Tamura, 2021; Basir *et al*, 2023; Brecht *et al,* 2023). These findings reinforce the mushroom's potential as a functional food in metabolic health management (Khan & Tania, 2015). The hypoglycemic effect of *P. tuber-regium* extract was evident across all treatment groups. Significant reductions in fasting blood glucose levels were observed over nine weeks, with the 400 mg/kg dose (PTM group) demonstrating the most pronounced effect (88.4% reduction). This aligns with earlier findings that oyster mushrooms exert a dose-dependent glycemic modulation, likely through bioactive compounds that enhance insulin secretion or activity and modulate glucose uptake and transport (Asrafuzzaman *et al*, 2018; Gochhi *et al*, 2024; Huang *et al*, 2012). Furthermore, the extract influenced pancreatic amylase (PA) activity, a key enzyme in starch digestion. Initially, a significant increase in PA activity was noted in the lower-dose (200 mg/kg), mid-dose (400 mg/kg), and standard drug (STD) groups at day 28, with the highest activity in the STD group. In contrast, the high-dose group (800 mg/kg) exhibited near-normal PA levels, suggesting a dose-dependent biphasic response (Kobayashi *et al*, 2021). By day 63, all treatment groups, except PTM and STD, showed reduced PA activity, indicative of enzyme regulation and potential restoration of pancreatic function (Kirk *et al*, 2006; Massani & Stecca, 2022). The initial elevation in PA may reflect a compensatory response to STZ-induced pancreatic damage, while the subsequent decline indicates recovery, potentially mediated by antioxidant and anti-inflammatory actions (Butterworth *et al*, 2011; *Sales et al*, 2011; Murtaugh & Keefe, 2015).

The extract's enzyme-modulating effects coincided with improved glycemic control, particularly in the PTM group. Given the interconnection between glucose and lipid metabolism in diabetes pathophysiology (Zhang *et al*, 2022; Bu *et al,* 2024), serum lipid profiles were also evaluated. After 28 days, significant reductions in total cholesterol were observed in both PTM and STD groups. By day 63, all treatment groups recorded lower total cholesterol levels compared to the diabetic control. However, high-density lipoprotein (HDL) cholesterol levels showed a less consistent pattern, with decreases noted in the PTM, PTH, and STD groups at day 28, and further reductions in PTH and STD by day 63. These trends suggest that higher doses of the extract may not support long-term maintenance of HDL cholesterol, possibly due to altered lipid transport mechanisms in diabetic conditions (Femlak *et al,* 2017). While the extract effectively reduced overall cholesterol, its role in promoting reverse cholesterol transport at higher doses remains questionable (Marques *et al*, 2018; Ouimet *et al*, 2019). Triglyceride levels significantly declined in PTM and STD groups by week 4 but rose in the high-dose group (PTH) by week 9, whereas they continued to decline in the PTE, PTM, and STD groups. Low-density lipoprotein (LDL) cholesterol was significantly elevated in the PTH group at day 28 but decreased across all groups by day 63.

Histological analysis of pancreatic tissues provided structural evidence supporting the biochemical results. The non-diabetic control group maintained intact islet architecture throughout the study, while treated diabetic groups (PTE, PTM, PTH, and STD) displayed mild to moderate regeneration of β-cell structures within the islets of Langerhans (Retnakaran *et al*, 2023; Li *et al*, 2024). These morphological improvements suggest that *P. tuber-regium* extract possesses pancreatic regenerative properties, potentially through antioxidant-mediated cytoprotection (Huang *et al,* 2012; Adeyi *et al*, 2021).

4. Conclusion

This study demonstrates persuasive evidence that the ethanolic extract of *P. tuber-regium* possesses nutritional and therapeutic potentials for improving nutritional status, diabetic, hypercholesterolemic conditions and pancreatic dysfunctions, induced with in high-fat-streptozotocin-induced Wistar male albino rats. The nutrient-rich mushroom, did not only support glycemic control but also regulated lipid and pancreatic amylase activity. Notably, the 400 mg/kg dose (PTM group) produced the most pronounced hypoglycemic and lipid-lowering effects, highlighting a dose-dependent therapeutic response. The extract's ability to regulate pancreatic amylase activity and promote histological regeneration of β-cell architecture further suggests its protective and restorative effects on pancreatic function. While reductions in total cholesterol and LDL levels were consistent across treatment groups, the less favorable impact on HDL levels at higher doses warrants further investigation. Overall, these findings highlight *P. tuber-regium* as a nutritionally rich and potentially safe nutraceutical or adjunctive therapy for metabolic disorders particularly diabetes, owing to its multifaceted biological activities and no side effects. Further clinical studies are recommended to validate these preclinical observations and establish standardized dosing regimens for therapeutic applications for managing type II diabetes and preserving pancreatic function.

**Disclaimer (Artificial Intelligence)**

Author(s) hereby declares that NO generative AI technologies such Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of the manuscript.

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